THE ELECTROKINETIC BEHAVIOR OF CALCIUM OXALATE MONOHYDRATE IN MACROMOLECULAR SOLUTIONS

P. A. Curreri*, G. Y. Onoda, Jr.**, and B. Finlayson***

*Space Science Laboratory/Code ES74, NASA/MSFC
Marshall Space Flight Center, AL 35812

**Watson Research Center, IBM, P.O. Box 218
Yorktown Heights, NY 10598

***Division of Urology, Department of Surgery, University of Florida,
College of Medicine, Gainesville, Florida 32610

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ABSTRACT

Electrophoretic mobilities were measured for calcium oxalate monohydrate (COM) in solutions containing macromolecules. Two mucopolysaccharides (sodium heparin and chrondroitin sulfate) and two proteins (positively charged lysozyme and negatively charged bovine serum albumin) were studied as adsorbates. The effects of pH, calcium oxalate surface charge (varied by calcium or oxalate ion activity), and citrate concentration were investigated.

All four macromolecules showed evidence for chemical adsorption. The macromolecule concentrations needed for reversing the surface charge indicated that the mucopopolysacchrides have greater affinity for the COM surface than the proteins. The amount of proteins that can chemically adsorb appears to be limited to approximately one monomolecular layer. When the surface charge is high, an insufficient number of proteins can chemically adsorb to neutralize or reverse the surface charge. The remaining surface charge is balanced by proteins held near the surface by longer range electrostatic forces only. Citrate ions at high concentrations appear to compete effectively with the negative protein for surface sites but show no evidence for competing with the positively charged protein.

I. INTRODUCTION

The majority of renal stones are predominantly calcium oxalate monohydrate and dihydrate, with the former being the most common form present. Interdispersed throughout a stone between the crystallizing phases is a macromolecular substance is typically around 2.5% (Boyce, 1968). It is not known whether the matrix substances play an active or passive role in the formation of urinary stones. However, it is known that several soluble polymeric species and natural macromolecules have a pronounced effect on the kinetics of growth of calcium oxalate crystals (Dent and Sutor, 1971; Nakagawa, Kaiser, and Coe, 1978). Such molecules can also affect the manner in which crystalline particles in suspension interact with each other. Adsorbed molecules can help prevent the coagulation (aggregation) of particles in suspension by providing an electrical barrier or steric hindrance (Vincent, 1974). Other molecules can function as flocculating agents, causing particles to be bridged together to form large flocs. Coagulation and flocculation phenomena provide one possible mechanism for creating larger units of matter from finely divided crystals.

The electrical charge residing on the surfaces of calcium oxalate crystals exposed to aqueous solutions should be strongly modified by the adsorption of charged ionic macromolecules. This effect has been reported for other solid surfaces such as silica (Dixon, La Mer, Casslon, Messinger, 1967), silver iodide (Vincent, Bijsterbosch, and Lyklema, 1971; Fleer, Koopal, and Lyklema, 1972), latexes (Norde and Lyklema, 1978), calcium phosphate (Healy and La Mer, 1964), etc. That certain macromolecules adsorb on calcium oxalate has been demonstrated (Leal and

Finlayson, 1977), but the effect on surface charge has not been examined in detail.

In a previous paper (Curreri, Onoda, and Finlayson, 1979), we described the effects of small ionic species on the surface charge of calcium oxalate monohydrate (COM). The effects were detected by measuring the electrophoretic mobility of the particles in the aqueous phase. The influences of activity of calcium and oxalate ions, monovalent electrolytes, sulfate, phosphate, pyrophosphate, and citrate ions on the electrophoretic mobility were studied. It was found that the results could be accounted for by certain established theories for the electrical double layer, which is also useful for analyzing the results of the present work.

In this investigation, we have used bovine serum albumin, lysozyme, sodium heparin, and chondroitin sulfate as adsorbates. Beside being of practical interest (Boyce and Swanson, 1955; Maxwell, 1963; Kentel and King, 1964), these macromolecules represent negatively charged proteins, positively charged proteins, and two distinct types of mucopolysaccharides.

II. METHODS

Commercially available* bovine serum albumin (2X crystallized), lysozyme (muramidase), sodium heparin, and chondroitin sulfate were used. The serum albumin and lysozyme are globular proteins with isoelectic points at pH 4.9 and 11, respectively. The sodium heparin and chondroitin sulfate are negatively charged mucopolysaccharides with random coil structures in solution. Other chemicals were of reagent grade. The water was deionized and then distilled in a borosilicate glass still. The

specific conductivity of the water was less than 1.5×10^{-6} $(\text{ohm cm})^{-1}$. All stock solutions were passed through a 0.22 μm filter to remove any undissolved impurities.

The calcium oxalate monohydrate (COM) crystals were precipitated by the method described in a previous paper (Curreri et al., 1979). Working suspensions were prepared by adding macromolecule solutions of varying concentration to suspensions of COM that had been equilibrated at least 12 hours after the desired addition of other simple electrolytes were made. The solids content of the suspensions was 0.315 g/l in all cases. The crystals had a nominal surface area of 3 m^2/g as measured by gas adsorption.

Before making electrophoresis measurements, all suspensions after final compositional adjustments were allowed to equilibrate for at least two hours at 37 °C. Electrophoresis was carried out using a commercial instrument† in a constant temperature chamber at 37 °C. The pH was measured with glass electrodes. For some of the suspensions, the protein concentrations remaining in solution were determined by eliminating the solids by filtration through a 0.22 μm filter and analyzing the filtrant for proteins using solution transmission spectroscopy at 280 nm wavelength.

include methods

* Nutritional Biochemicals Inc. Cleveland, Ohio.

† Zeta Meter, Inc., New York, N.Y.

III. RESULTS

The changes in the electrophoretic mobility of COM when increasing concentration of the four macromolecules are present are shown in Fig. 1. In the previous study it was shown that the charge on calcium oxalate in its own natural saturated solution is positive and constant throughout a broad pH range (pH 4-10). In Fig. 1 it is seen that the two mucopolysaccharides cause a reversal in charge at relatively low solution concentrations. The serum albumin reverses the charge only at higher concentrations. The lysozyme appeared to have no tendency for change reversal.

Because of the low solid content of the suspensions, there was not enough surface area present to expect detectable solution depletion as a result of adsorption. This was confirmed in the case of the proteins by measuring their concentrations in solution before and after exposure to the solid. Also there was no evidence of any precipitation reactions involving the macromolecules.

The effect of pH on the electrophoretic mobility of COM in systems containing fixed amounts of the four macromolecules is shown in Fig. 2. Higher pH values in general led to more negative values of the electrophoretic mobility. The most marked effect was with serum albumin, where a reversal of charge occurred near pH 5.5.

The activity of calcium and oxalate ions in solution has been shown to strongly affect the electrophoretic mobility of COM (Curreri et al., 1979). A surface isoelectric point was found at a pCa of 5.2 (corresponding to a pC_2O_4 of 3.45). It follows that for calcium ion activities above that of the isoelectric point, the surface charge of COM

is positive, and for oxalate activities above that for the isoelectric point, the surface is negatively charged. The data in Fig. 1 were obtained under conditions where the surface of COM is normally positively charged. The absence of any noticeable effect of lysozyme on surface charge might be attributable to the fact that the charge on the lysozyme has the same sign as the surface. For the above reasons, we were interested as to whether the lysozyme would adsorb to the COM surface if the surface had been negatively charged to begin with. In our previous work, we showed that a negative surface charge is brought about by increasing the oxalate activity in solution. Thus, to accomplish this, different strengths of lysozyme were added to COM suspensions that were equilibrated with sodium oxalate. In Fig. 3 it is seen that increasing concentrations of lysozyme reduces the negative mobility of the originally negative surfaces. The negative mobility is reduced to near zero at high lysozyme concentrations.

For the adsorption of the two proteins, the role of surface charge due to variations in the concentrations of the potential determining ions (calcium and oxalate ions) was investigated further. In Fig. 4, the mobility of COM suspensions containing either of the two proteins is given as a function of the calcium and oxalate concentrations. These concentrations were varied by additions of calcium chloride or sodium oxalate. The change in mobility for COM suspensions without macromolecules present is shown with the solid data points.

In the previous paper, it was shown that citrate ions adsorb strongly onto COM. It was of interest to determine how this relatively simple species would perturb the electrokinetic response of suspensions containing the two proteins. The mobility for suspensions containing fixed concentrations of the two proteins as a function of the concentration of

added sodium citrate is given in Fig. 5. These are compared with the results found in suspensions containing no proteins. In all three cases, it was found that increasing concentration of sodium citrate resulted in increasing negative mobility.

IV. DISCUSSION

The development of increasingly negative mobilities of COM with three of the added macromolecules, Fig. 1, can result from one of two mechanisms. One is the adsorption of the negatively-charged macromolecules. The second possibility is that the macromolecules bind calcium in solution and cause the increase on oxalate activity, which in turn would cause the mobility to become more negative. This second mechanism does not seem plausible, however, because of the increase in the oxalate activity that would be required to account for the observed changes in mobility. Based on the previous work, it would require more than 0.01 molar oxalate ions in solution to bring about a COM mobility reversal from +1.7 to -1.7 mobility units. The amount of solids in the suspension is not sufficient, even if all were to dissolve, to produce 0.002 molar oxalate. In addition, the magnitude of calcium binding by proteins indicated by values given in the literature (Munday and Mahy, 1964; Blatt and Robinson, 1968) would also not be enough of a depletion of calcium activity to appreciably affect the COM mobility. Thus, it appears that the adsorption mechanism is the more likely of the two alternatives.

We see in Fig. 1 that the two mucopolysaccharides adsorb strongly onto the positively charged solid. The less negatively charged serum albumin adsorbs weakly, whereas the positively charged protein (lysozyme) shows no effect. On an originally negative surface, as produced by oxalate

addition, the lysozyme has a small effect on making the surface more positive with increasing concentrations (Fig. 3). Some specific adsorption tendency is suggested by the small charge reversal that takes place for the intermediate sodium oxalate concentration.

It was shown (Curreri et al., 1979) that the mobility of COM remained unaffected over a pH range of 4 to 10. However, changes in pH are known to alter the net charge on the macromolecules. If surface coverage is relatively high, changes in the charge of adsorbed polymer molecules due to pH variations should be reflected in the electrophoretic mobility. In fact, this assumption is often made in the study of charges on macromolecules adsorbed on glass capillaries (Shaw, 1979). The variations in mobility exhibited in Fig. 2 qualitatively follow what is expected for the changes in the charge of the macromolecules. The isolectric points based on mobility can be estimated by extrapolation or interpolation of the curves. These values agree closely with the known isoelectric points of the macromolecules (Cohn, Hudges, and Weare, 1947; Anderson and Alberty, 1948).

The nature of the adsorption process at high solution concentration of proteins appears to be particularly interesting. From Fig. 4, it can be inferred that, when the surface originally has a high charge (positive or negative), the adsorption of relatively high concentrations of a protein having an opposite charge from the surface occurs in a manner that reduces the mobility to zero. When the activity of calcium ions is high (giving a high positive charge to COM), the negatively charge protein adsorbs to an extent that reduces the mobility to zero. Similarly, at high oxalate activity (a negative surface charge), adsorption of positively charged protein reduces the COM mobility to zero. In simple double layer theory,

it is not expected that chemical adsorption of ions of opposite charge to the surface would produce zero mobility (Overbeek, 1952). The mobility should change continuously and at some point specific adsorption should cause a reversal in charge.

A possible explanation for the apparent anomaly is that the surface is being saturated by the adsorbing proteins. The charge on the surface is too high to be completely balanced by a complete monomolecular layer of proteins. The remaining charge excess must be balanced by proteins that are further out from the first monomolecular layer. These molecules are simply attracted by the long-range electric field due to the remaining surface charge (e.g., as counter ions). The counter ions, because of their lack of chemical affinity, cannot contribute to a reversal of charge, but can only help reduce the charge to zero.

To test the above hypothesis, direct measurements of bovine serum adsorption by COM reported by Leal and Finlayson were analyzed. They presented adsorption isotherms at several calcium ion activities. It was found that individual isotherms fit the general form of the Langmuir isotherm. However, the calculated maximum adsorption density (saturation) was found to vary proportionally with the log of the activity of calcium ions in solution. This dependence, which the Langmuir model does not consider, requires a more advanced adsorption isotherm to explain. However, since for any particular calcium concentration the Langmuir model apparently describes the empirical results, we used Langmuir adsorption parameters implied by Leal and Finlayson's work to calculate the adsorption densities for our experimental conditions.

The total projected area of protein adsorbed at each calcium concentration can be estimated and compared to the total surface area

available for adsorption. If we assume that an adsorbed serum albumin molecule can be approximated by a sphere with molecular weight 69,000 and density 1.37 gm/ml, then the projected area per molecule adsorbed is calculated to be around 23 ηm^2. The adsorption density begins to approach that of closed packed spheres at 10^{-3} mol/l calcium concentration and exceeds the total available calcium oxalate surface area at between 10^{-3} and 10^{-2} mol/l calcium concentration. This corresponds with the calcium ion concentration in Fig. 4 above which the mobility is fixed at zero. It suggests, therefore, that at higher calcium ion concentrations the adsorption density would exceed one monolayer, thus supporting the multilayer hypothesis.

The studies with citrate provide some information on competition between a stongly adsorbing small molecule and a macromolecule. It might be expected that if molecules compete for sites on the surface, the addition of higher concentrations of citrate should lead to a reduction in the amount of protein adsorbed. We find in Fig. 5/6 that at low citrate concentrations the presence of the negatively charged protein causes the mobility to be much more negative than if only the citrate were present. However, when the citrate concentration is large, the presence of the protein has no effect. At 10^{-2} mol/l citrate, the ratio of citrate to protein molecules in solution is around one thousand, whereas the same ratio at 10^{-4} mol/l citrate is only ten. It appears that the presence of high concentrations of citrate prevent appreciable adsorption of the negatively charged protein. In contrast, the positively charge protein always has the effect of providing a more positive surface, irrespective of the amount of citrate in the system. Citrate apparently does not interfere with the adsorption of the lysozyme, suggesting that they are

not competing for the same surface sites.

V. CONCLUSIONS

Two mucopolysaccharides (sodium heparan and chondroitin sulfate) adsorb strongly onto calcium oxalate. A negatively charged protein, bovine serum albumin, adsorbs weakly onto positively charged calcium oxalate surfaces. A positively charged protein, lysozyme, adsorbs weakly on negatively charged calcium oxalate, as produced by adjustment in the oxalate activity.

The adsorption mechanism of proteins appears to depend on the magnitude of the surface charge. With low surface charge, the charge can be balanced by proteins adsorbing within the first monomolecular layer. However, if the surface is highly charged, the charge cannot be balanced by a complete monolayer of proteins. After a monolayer is formed, other molecules are still electrostatically attracted into a seond layer by the remaining unsatisfied surface charge. With this crowding effect, the proteins cannot reverse the charge on calcium oxalate because the specific adsorption sites on the crystal surface are no longer available to the proteins beyond a monolayer.

Citrate ions at high concentrations appear to effectively compete with the negative protein for surface sites. They show no evidence for competing with the positively charged protein.

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FIGURE CAPTIONS

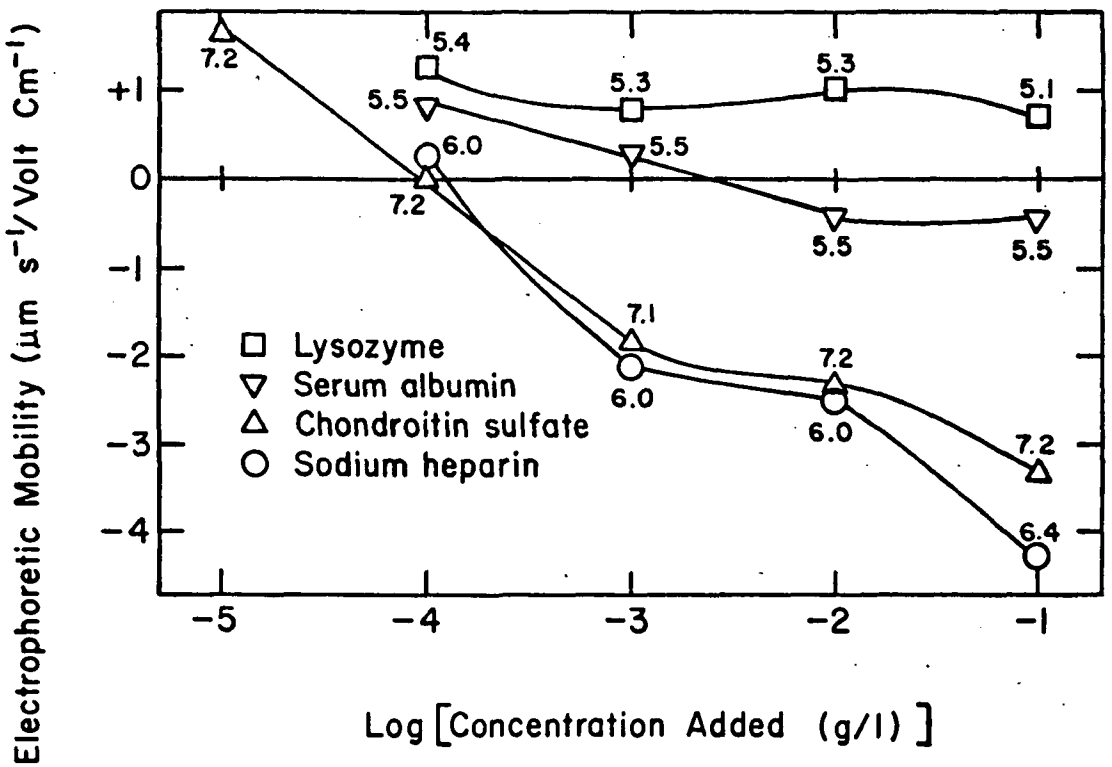
Fig. 1. Electrophoretic mobility of calcium oxalate monohydrate versus macromolecule concentration. The numbers near the data points are the corresponding solution pH values.

Fig. 2. Electrophoretic mobility of calcium oxalate monohydrate with 0.1 g/l of macromolecule as a function of solution pH. The dashed curve without data points represents the mobility versus pH without macromolecules present.

Fig. 3. Electrophoretic mobility of calcium oxalate monohydrate versus lysozyme concentration for different sodium oxalate concentrations. The numbers near the data points are solution pH. The left most data points are mobilities without lysozyme.

Fig. 4. Electrophoretic mobility of calcium oxalate with 0.1 g/l macromolecule for various calcium chloride or sodium oxalate additions. The numbers near the data points are solution pH.

Fig. 5. Electrophoretic mobility of calcium oxalate monohydrate with 0.1 g/l proteins versus citrate concentration. The numbers near the data points are solution pH. The left most data points are for zero citrate concentrations.


Electrophoretic Mobility (μm s⁻¹/Volt Cm⁻¹)
+1
0
-1
-2
-3
-4
-5
-4
-3
-2
-1
Log [Concentration Added (g/l)]
Lysozyme
Serum albumin
Chondroitin sulfate
Sodium heparin
7.2
5.4
5.3
5.3
5.1
5.5
6.0
5.5
7.2
5.5
5.5
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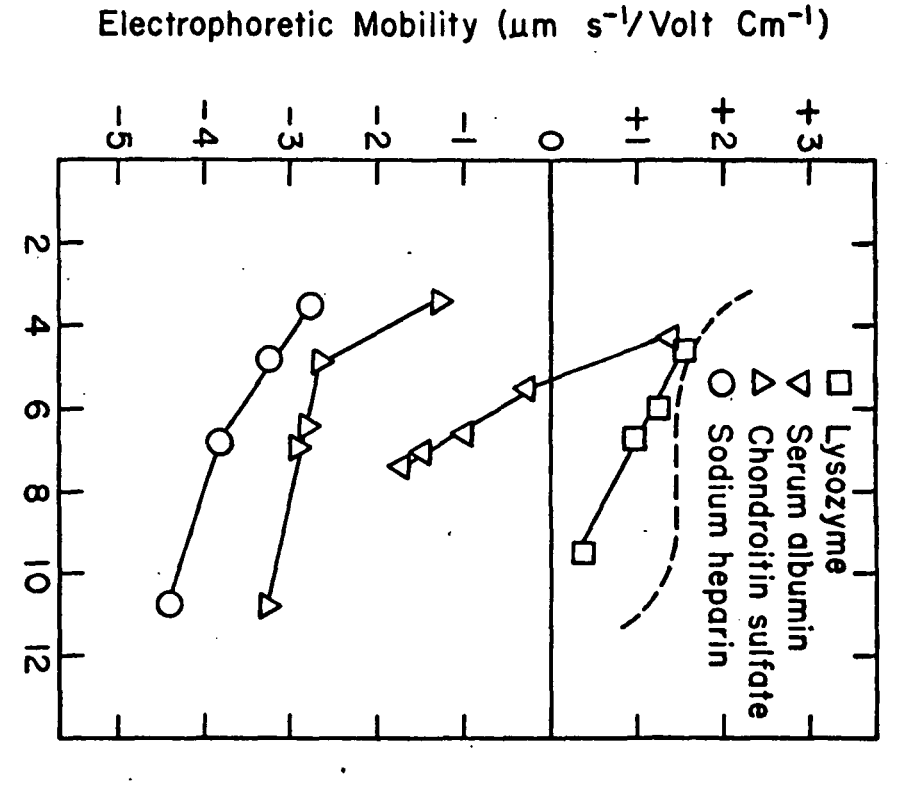
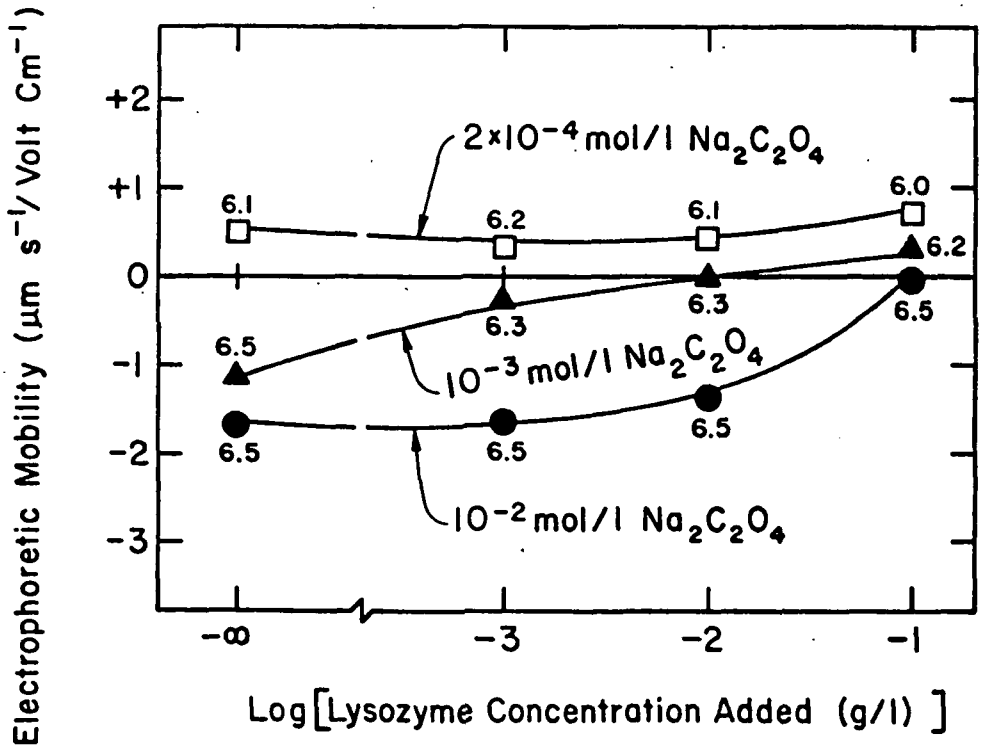
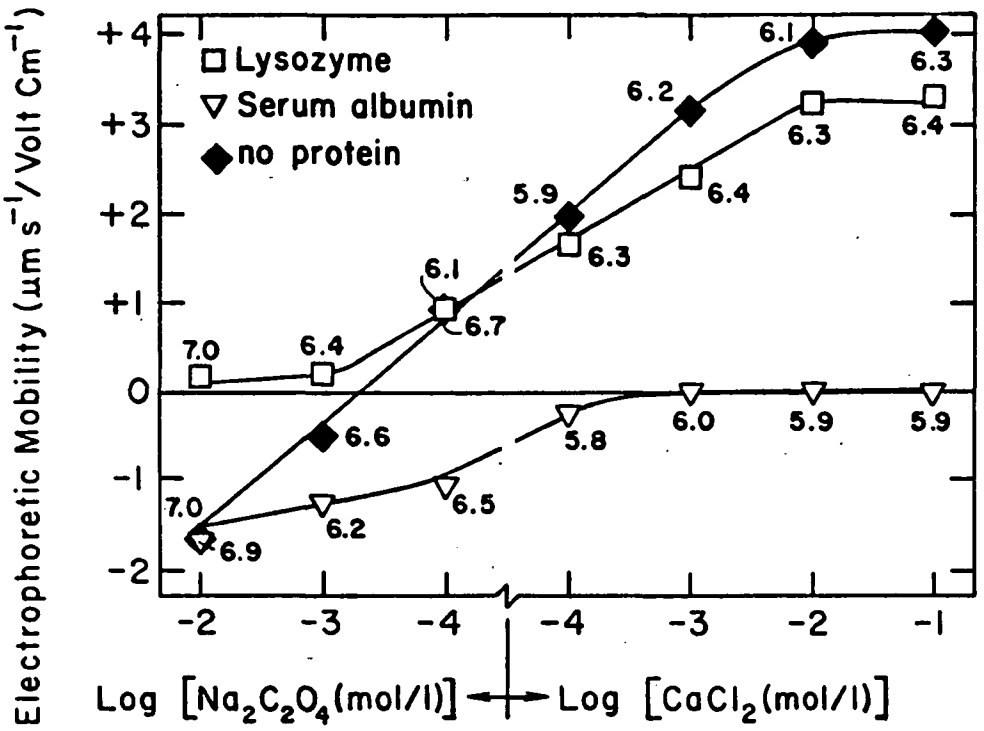
7.2
6.0
6.0
7.2
6.4

(2)

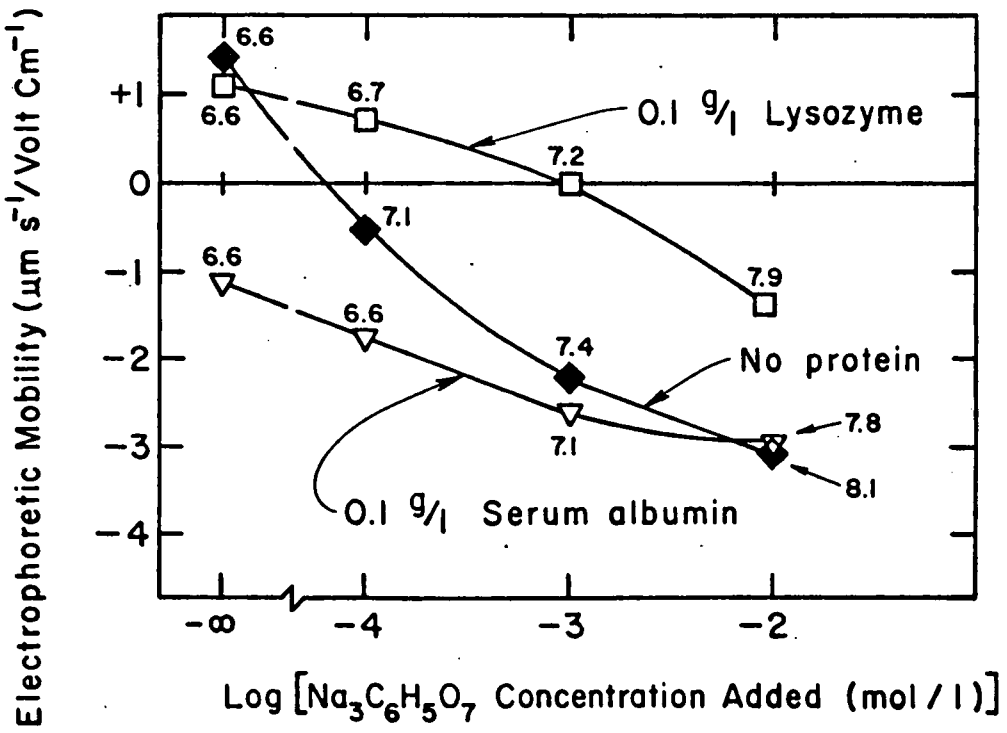

Electrophoretic Mobility (μm s⁻¹/Volt Cm⁻¹)
-5
-4
-3
-2
-1
0
+1
+2
+3
pH
2
4
6
8
10
12
Lysozyme
Serum albumin
Chondroitin sulfate
Sodium heparin

(3)


Electrophoretic Mobility (μm s⁻¹/ Volt Cm⁻¹)
+2
+1
0
-1
-2
-3
2×10⁻⁴ mol/l Na₂C₂O₄
10⁻³ mol/l Na₂C₂O₄
10⁻² mol/l Na₂C₂O₄
6.1
6.2
6.1
6.0
6.2
6.3
6.3
6.5
6.5
6.5
6.5
6.5
-∞
-3
-2
-1
Log [Lysozyme Concentration Added (g/l)]


Electrophoretic Mobility (μm s⁻¹/Volt Cm⁻¹)
+4
+3
+2
+1
0
-1
-2
Lysozyme
Serum albumin
no protein
7.0
6.4
6.1
6.7
6.6
5.9
6.3
6.2
6.1
6.3
6.4
6.4
6.3
7.0
6.9
6.2
6.5
5.8
6.0
5.9
5.9
-2
-3
-4
-4
-3
-2
-1
Log [Na2C2O4(mol/l)]
Log [CaCl2(mol/l)]

(4)


Electrophoretic Mobility (μm s⁻¹/Volt Cm⁻¹)
+1
0
−1
−2
−3
−4
−∞
−4
−3
−2
Log [Na3C6H5O7 Concentration Added (mol/l)]
6.6
6.6
6.7
7.2
7.9
0.1 g/l Lysozyme
7.1
6.6
6.6
7.4
No protein
7.8
7.1
8.1
0.1 g/l Serum albumin

Keywords: calcium oxalate, urolithiasis, surface chemistry, macromolecular adsorption.